PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Eric N. OLSON	Group Art Unit: 1632
Serial No.: 10/043,658	Examiner: Woitach, Joseph T.
Filed: January 9, 2002	Atty. Dkt. No.: MYOG:024USC1/SLH
For: METHODS FOR PREVENTING HYPERTROPGY AND HEART FAILURE BY INHIBITION OF MEF2 TRANSCRIPTION FACTOR	

CERTIFICATE OF MAILING
37 C.F.R. § 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-01450, on the date below:

May 9, 2005
Date

Steven L. Highlander

DECLARATION OF TIM MCKINSEY UNDER 37 C.F.R. §1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-01450

Dear Sir:

I, Tim McKinsey, do declare the following:

1. I currently hold the position of Scientist II at Myogen, Inc., licensee of the above-captioned application. I also hold academic appointments in the Division of Cardiology at the University of Colorado Health Sciences Center and in the Department of Molecular Biology at the University of Colorado in Boulder. My education and training includes an undergraduate degree in Biological Sciences from University of Missouri and a Ph.D. in

Microbiology and Immunology from Vanderbilt University School of Medicine. I completed four years of post-doctoral training in the Department of Molecular Biology at the University of Texas Southwestern Medical School prior to moving to Colorado. I have published numerous original research papers and multiple reviews on the molecular mechanisms controlling heart muscle disease. In addition, I have given many invited lectures on the topic at universities and at national and international scientific meetings. For the past two years, I have been exclusively engaged in the discovery and validation of molecular drug targets for use in drug discovery in the field of heart failure. I am intimately familiar with the studies of MEF2 and Class II HDACs.

2. I am also familiar with the level of skill of scientists working in the field of cardiology and molecular biology as of the priority date of the referenced application. I consider one of ordinary skill in the art in this field of study to have a Ph.D. in biochemistry, chemistry, molecular biology, pathology or other related field, or an M.D., with 1-3 years of post-graduate study.

3. I have reviewed the specification and pending claims 1, 4, and 9 for the above-referenced case. The application describes and then subsequently claims the inhibition of MEF2 or MEF2 dependent gene transcription as a treatment for hypertrophy. I have also reviewed the examiner's assertions that the current patent specification does not give sufficient guidance for one of skill in the field of cardiac biology to practice the invention without having to undertake extensive experimentation.

4. The inventors' paradigm, as defined through the *in vitro* and *in vivo* models, is that in healthy heart tissue MEF2 is bound to and inactivated by Class II HDACs. A stress

signal or response leads to dissociation of this complex, followed by activation of a "fetal gene" cascade that invariably leads to hypertrophy. Inhibiting MEF2 dependent gene transcription, inhibiting MEF2 itself, or inhibiting the upregulation of a gene that is regulated by MEF2, is therefore considered anti-hypertrophic by the inventors.

5. I have reviewed the enclosed article by Zhang *et al.*, entitled "Class II Histone Deacetylases Act as Signal-Responsive Repressors of Cardiac Hypertrophy," *Cell*, 110:479-488 (2002), and have supplied my own review article McKinsey *et al.*, entitled "MEF2: a calcium-dependent regulator of cell division, differentiation and death," *TRENDS Biochem. Sci.*, 27:40-47 (2002), both of which support the inventor's claims relating to inhibition of MEF2 as a therapeutic target, and further explain the interaction between MEF2 and Class II HDACs in relation to cardiac hypertrophy. In Zhang *et al.*, the authors wanted to determine whether earlier reports of class II HDACs being potent repressors of MEF2 were indeed valid. The paper provides *in vivo* evidence that unphosphorylated Class II HDACs associate with and repress MEF2, and that pro-hypertrophic stimuli lead to phosphorylation-dependent release of class II HDACs from MEF2. Once HDACs are released, MEF2 can and does initiate transcription of fetal genes, leading to the development of hypertrophy. Signal resistant HDACs (see Fig. 2A) that are incapable of being phosphorylated are constitutive repressors of MEF2-dependent transcription and cardiac hypertrophy. The fact that these dominant repressive HDACs are anti-hypertrophic is not only validation of a role for HDACs in hypertrophy, it also shows direct proof that inhibiting MEF2 and MEF2 dependent gene upregulation is anti-hypertrophic. The reference also shows (See Fig. 4) that knocking out a Class II HDAC leads to loss of MEF2 regulation and development of profound and rapid cardiac

hypertrophy. These experiments are direct evidence and proof that inhibiting MEF2 would be a viable therapeutic regimen for the treatment of cardiac hypertrophy.

Additionally, McKinsey *et al.*, which is a review of the state of the art, further explains the interaction between MEF2 and class II HDACs and goes on to highlight the importance of MEF2 transcriptional activation as a key feature of a variety of cellular cascades. It is true that it has not been conclusively shown that direct inhibition of MEF2 ablates hypertrophy, those experiments have yet to be done, but this article addresses the importance of MEF2 and its interaction with not just class II HDACs, but calcineurin phosphatase and the CaM Kinases, all of which have been implicated as major players in hypertrophy. Read together, along with the specification, these articles demonstrate and validate a treatment for cardiac hypertrophy involving the inhibition of MEF2 or MEF2 dependent gene transcription.

6. I hereby declare that all statements made of my own knowledge are true and all statements made on information are believed to be true and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

4-4-05
Date

Timothy A. McKinsey, Ph.D.